American Journal of Medical Genetics 92:69–76 (2000)

Mosaic Tetrasomy 8q: Inverted Duplication of 8q23.3qter in an Analphoid Marker

Kavita S. Reddy,[1]* Vladimira Sulcova,[1] Stuart Schwartz,[2] Julie E. Noble,[3] Jeffrey Phillips,[4] Jo Anne Brasel,[3] Kenneth Huff,[3] and Henry J. Lin[3]

[1]*Cytogenetics Laboratory, Quest Diagnostics Inc., San Juan Capistrano, California*
[2]*Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio*
[3]*Department of Pediatrics, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, California*
[4]*Department of Radiology, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, California*

We observed an analphoid marker chromosome stable through cell division in a 16-year-old girl with developmental delay, short stature, limb contractures, and ovaries containing multiple cysts. She also developed myasthenia gravis at 15 years. The marker chromosome, present in 75% of metaphases (and in 90% of transformed lymphoblastoid cells), was C-band negative, and had no pan α-satellite sequences detectable by fluorescence in situ hybridization (FISH). The 8q origin of the marker was determined by use of subtelomeric probes and was confirmed by chromosome 8 painting probes. The marker was shown to be an inversion duplication of 8q when subtelomeric, telomeric, and c-myc FISH probes hybridized to both ends of the marker. The karyotype was 47,XX,+inv dup(8)(qter→q23.3::q23.3→[neocen]→qter), resulting in tetrasomy for 8q23.3qter. The parents had normal karyotypes. Centromeric proteins CENP-C and CENP-E were present, but alpha associated centromere protein CENP-B was absent at a position defining a neocentromere. Am. J. Med. Genet. 92:69–76, 2000.

KEY WORDS: analphoid marker chromosome; tetrasomy 8q23.3qter; neocentromere

INTRODUCTION

Supernumerary marker chromosomes occur in 0.05% of live births [Buckton et al., 1985]. Most of these markers have α-satellite sequences detectable by fluorescence in situ hybridation (FISH) [Callen et al., 1992], but some lack C-positive alphoid sequences [reviewed in Choo, 1997]. These analphoid chromosomes have changed our understanding of centromeres, giving rise to the concept of neocentromeres. A newly derived functional centromere that is formed outside the normal centromere domain is a neocentromere (Slater et al., 1999).

At least 38 markers with a neocentromere have been reported in the literature, representing chromosomes 1, 2, 3, 8–11, 13–15, 19, 20, and Y. Because the markers originate from many different and apparently unique parts of the genome, there appear to be many centromere-competent sites, possibly formed by epigenetic modification of chromatin [Barry et al., 1999; Karpen and Allshire, 1997; Vafa and Sullivan, 1997; Warburton et al., 1997b]. Most analphoid markers are inversion duplications, frequently involving chromosomes 13 or 15. These chromosomes have two centromere-competent sites, but only one of the sites forms an active neocentromere, reminiscent of pseudodicentric chromosomes [Faulkner et al., 1998; Page et al., 1995]. Ohashi et al. [1994] reported an analphoid inversion duplication of the short arm of chromosome 8. Here we add to the list of known neocentromeres by describing a new analphoid marker derived from the long arm of chromosome 8.

CLINICAL REPORT

The patient was a 16-year-old African American girl. She was born to a 31-year old primigravid mother following a 32-week pregnancy and uncomplicated vaginal delivery. The mother reportedly drank six to seven quarts of beer per day for two to three years and throughout the pregnancy and smoked one to two packs of cigarettes per day. She also had a psychiatric history, including treatment with trifluoperazine and chlorpromazine. Birth weight was 1510 g, and Apgar scores were 7 at one minute and 8 at five minutes. Urine screening for amphetamines, barbiturates, benzodiazepines, opiates, cocaine, and phencyclidine was negative.

*Correspondence to: Dr. Kavita S. Reddy, Cytogenetics Department, Quest Diagnostics Inc., San Juan Capistrano, CA 92690. E-mail: reddyk@questdiagnostics.com

Received 28 October 1999; Accepted 12 January 2000

Gavage feeding was needed for poor suck. Examination at 20 days showed a weight of 1720 g, a length of 43 cm, an OFC of 29 cm (all measurements <5th centile), increased hair on the forehead, camptodactyly of the 5th fingers, rocker bottom feet, and hypertonia with tight fisting of the hands and limited extension of the elbows, knees, and hips. An echocardiogram, ophthalmology examinations, and brainstem-evoked responses were judged to be normal. The chromosome constitution was 47,XX with a nonsatellited, C-band negative marker of unknown origin in all 24 cells studied. Parental karyotypes were normal.

Weight and height were below the 5th centile throughout development. A genetic skeletal survey at 2-3/12 years showed a small skull and normal spine and limbs. Bone age was compatible with 2 years (with a standard deviation of ±4.6 months).

Development was delayed. She sat at 1 year and walked at 1-1/2 years. Psychological testing at 5 years showed a full-scale IQ score of 45 (verbal 52; performance 40; Wechsler Preschool and Primary Scale of Intelligence). Vineland Scales of Adaptive Behavior gave an age-equivalent score of 2.9 years, compatible with moderate mental retardation. Stanford-Binet testing (4th edition) at 7-4/12 years showed a composite IQ score of 50. By 10-10/12 years, she knew colors, the alphabet, and the flag salute. She was affectionate and social, but her teacher described her as difficult to control, aggressive, and having an attention deficit at age 12-11/12. A magnetic resonance image (MRI) of the brain at 15-5/12 years showed mild prominence of the ventricles and a few nonspecific foci of abnormal signal in the periventricular white matter bilaterally. When seen at 15-9/12, she could count to 27, was unable to read, and could undress but needed help putting clothes on.

There was persistent spasticity in the lower limbs, with tight hamstring muscles and heel cords. Casting of the knees and ankles was started at 9-6/12 years, because she walked on her toes. However, treatment was stopped due to problems with follow-up.

The patient developed facial hirsutism at 13 years with the onset of puberty and had four days of menstrual bleeding one month. An episode of "spotting" occurred one year later, and she had six days of menstrual bleeding one month at 15-8/12 years. Hormone levels at age 14-7/12 years were: thyroxine, 7.5 μg/dL (reference 4.5–12.0); thyroid stimulating hormone, 0.32 μU/mL (reference 0.39–4.6); total testosterone, 0.6 ng/mL (reference for late prepubertal females 0.1–0.2); estradiol, 119 pg/mL; luteinizing hormone, 19.1 mIU/mL; and follicle stimulating hormone, 13.6 mIU/mL. An MRI at 15-2/12 years showed elongated ovaries containing multiple cysts dispersed throughout (Fig. 1; the image was not considered typical of polycystic ovary syndrome, which usually shows cysts at the periphery). Kidneys were normal in size, with the right kidney being mildly malrotated.

At 15-1/12 years, she complained of drooping eyelids, double vision, bumping into objects, and some difficulty swallowing, all worsening over one week. An anticholinesterase test with edrophonium bromide (Tensilon) was compatible with myasthenia gravis. The anti-acetylcholine-receptor antibody level was 7.5 nmol/L (reference <0.5). She was treated with pyridostigmine, but side effects occurred, and prednisone was needed.

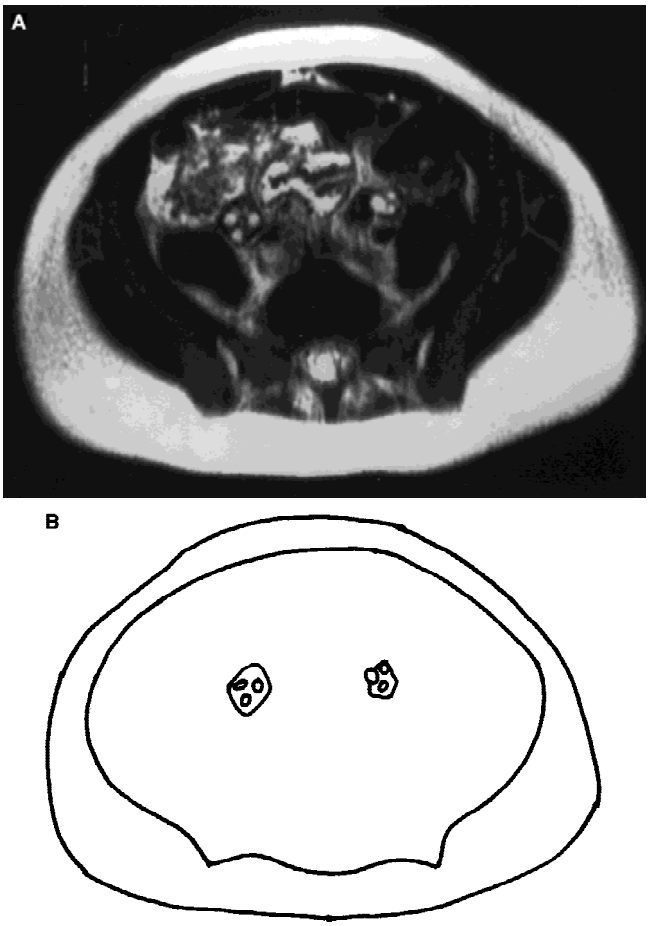

Fig. 1. MRI of ovaries. **A:** Transverse T2-weighted MRI (single shot fast spin echo) through the pelvis showing ovaries with multiple cysts or follicles of varying size (<1 cm diameter) scattered throughout. **B:** Diagram showing positions of ovaries.

Examination in the Genetics Clinic at age 15-9/12 showed: weight 41.4 kg (<5th centile; 50th centile for 12 years), height 131 cm (<5th centile; 50th centile for 7-1/2 years), OFC 56 cm (75th centile), inner canthal distance 3.2 cm (75–97th centile), ears 4.7 cm (<3rd centile; 50th centile for 9 months), hands 14.5–15 cm (<3rd centile; 50th centile for 9 years), palms 9 cm (3rd centile), and feet 19.5–20 cm (<3rd centile; 50th centile for 8 years). Facial hair was increased. There were contractures of the second, third, fourth, and fifth digits of mainly the right hand.

At 16 years, there was persistent ptosis, no lateral gaze, and no prolonged upward or downward gaze. At 16-4/12, there was a sixteen-day hospitalization for respiratory failure, and she was treated with plasmapheresis for antibody removal. Thymectomy was performed three months later, and no thymoma was found. The patient improved, allowing the prednisone dose to be reduced.

CYTOGENETIC STUDIES

Peripheral blood cultures were synchronized with methotrexate (amethopterin 0.05 μg/mL; Sigma; St. Louis, MO) for 17 hours and released with thymidine (2.5 μg/mL; Sigma) for 5.5 hours. Cultures were harvested after the addition of colcemid, and the chromosomes were GTG- and CBG-banded.

FISH studies were performed using coatasome 8, all human centromeric, chromosome 8 α-satellite sequence D8Z2, c-myc, all telomeric (previously available from Oncor, Gaithersburg, MD), and subtelomeric probes (Cytocell, Inc.). Slides were denatured in 70% formamide/ 2X SSC at 70°C for 2 min, followed by dehydration in 70, 80, 90% and absolute alcohol. Ten microliters of each probe was applied to the slides, which were cover-slipped and sealed. Following incubation overnight in a humid chamber at 37°C, the slides were washed in 2X SSC (all-human telomeres and c-myc probes), 1X SSC (coatasome 8 and all human centromere probes), or 0.25X SSC (D8Z2) at 72°C for 5 min. Detection was carried out using rhodamine labeled antidigoxigenin and 4,6-diamidino-2-phenylindole (DAPI) as counterstain. Subtelomeric probes for all chromosomes distributed one chromosome per grid (Cytocell, Inc.) were used. Two microliters of the cell pellet was spread into each grid. The metaphases were denatured on a hot plate at 70° for 2 min. The slide was hybridized overnight in a humid chamber at 37°C, then washed in 0.4X SSC at 72°C for 2 min, followed by 2X SSC and 0.05% Igepal (Sigma) for 30 sec. The p-subtelomere probe labeled with dioxygenin was detected with fluorescein isothiocyanate. The q-subtelomere probe labeled with biotin was detected with Cy3.

Polyclonal antibodies to CENP-B, CENP-C, and CENP-E were obtained from Drs. Huntington Willard (Case Western Reserve University, Cleveland, Ohio) and John Harrington (Athersys+, Cleveland, Ohio). Suspension cultures of cells were used to obtain unfixed metaphase preparations for immunofluorescence. A modification of the technique developed by Earnshaw et al. [1989] was used for the detection of CENP-B, CENP-C, and CENP-E [Sullivan and Schwartz, 1995]. Lymphoblastoid cells were grown on polylysine-coated slides and harvested as monolayer cell cultures. CENP antibodies were detected with secondary antibodies: fluoresceinated goat antirabbit (for CENP-B and -C antibodies) and rhodamine-conjugated goat antimouse (for CENP-E antibodies). Digital images were captured using a Zeiss epifluorescent microscope (Axioplan) equipped with a cooled CCD camera (Photometrics).

The supernumerary marker was found in 75% (15/20) of G-banded metaphases. The marker was about the size of chromosome 20 (Fig. 2A) and lacked a C-band (Fig. 2B). FISH investigation was initiated with an all centromeric probe, which did not give a signal on the marker (Fig. 3A). The chromosome origin of the marker was established using the subtelomeric probes in a grid system. The 8q subtelomeric probe (Fig. 3B) hybridized to the two ends of the marker. The all telomeric probe hybridized to the two ends of the marker

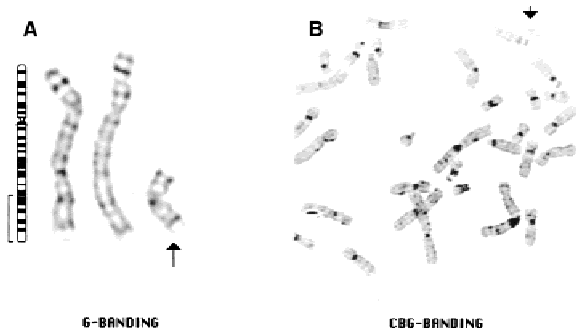

Fig 2. **A:** GTG-banded partial karyotype. From left to right, the figure shows an ideogram of chromosome 8 (at 850 band level with the duplicated segment indicated by a bracket), a normal chromosome 8 pair, and the supernumerary inv dup(8)(q23.3→qter) **(arrow)**. **B:** C-banded metaphase showing the supernumerary, acentric marker **(arrow)**.

(Fig. 3C), but the 8-centromere probe (DS8Z1) did not give a signal (Fig. 3D). Coatasome 8 probes painted the marker from end to end (Fig. 3E). There were two signals on the marker for the c-myc probe that maps to 8q24 (Fig. 3F). FISH studies showed that the marker did not have α-satellite sequences.

The FISH and G-band studies thus characterized the marker to be a mirror image duplication of 8q23.3qter. The active neocentromere appeared to be off center. The karyotype was mos 47,XX,+inv dup(8)(qter→q23.3::q23.3→[neocen]→qter)[15]/46,XX[5] and in 117 metaphases from a c-myc FISH slide 64 (55%) metaphases had a marker. A lymphoblastoid cell line had the analphoid marker in 90% of cells. Repeat karyotypes of both parents were normal. The neocentromere contained CENP-C and CENP-E but not CENP-B (Fig. 4A–C).

DISCUSSION

Trisomy 8 was first observed by Bijlsma et al. [1972] and Caspersson et al. [1972], and 17 other cases were identified over the next three years [Cassidy et al., 1975]. Partial trisomy for the long arm of chromosome 8 (8q24) was reported by Sanchez and Yunis [1974] in a translocation to the short arm of chromosome 22. Some of the clinical findings were similar to those of trisomy 8. Among the many cases of partial trisomy 8q, at least 12 involved 8q23qter [Romain et al., 1989; Stengel-Rutkowski et al., 1992].

To our knowledge, our patient represents the first report of tetrasomy for the long arm of chromosome 8 (8q23.3qter). Clinical findings included developmental delay, short stature, and contractures of the limbs and fingers, which are frequently associated with trisomy 8q [Stengel-Rutkowski et al., 1992]. The patient also had cystic ovaries with amenorrhea and hirsutism, which may represent polycystic ovary syndrome [Govind et al., 1999]. However, the ovaries seen by MRI did not have the appearance typical of the syndrome [Taylor, 1998]. Abnormal ovaries could be part of a trisomy 8q or tetrasomy 8q complex. For example, two girls (ages 6 and 12 years) with trisomy 8q have been described as hirsute [Fryns et al., 1974; Romain et al.,

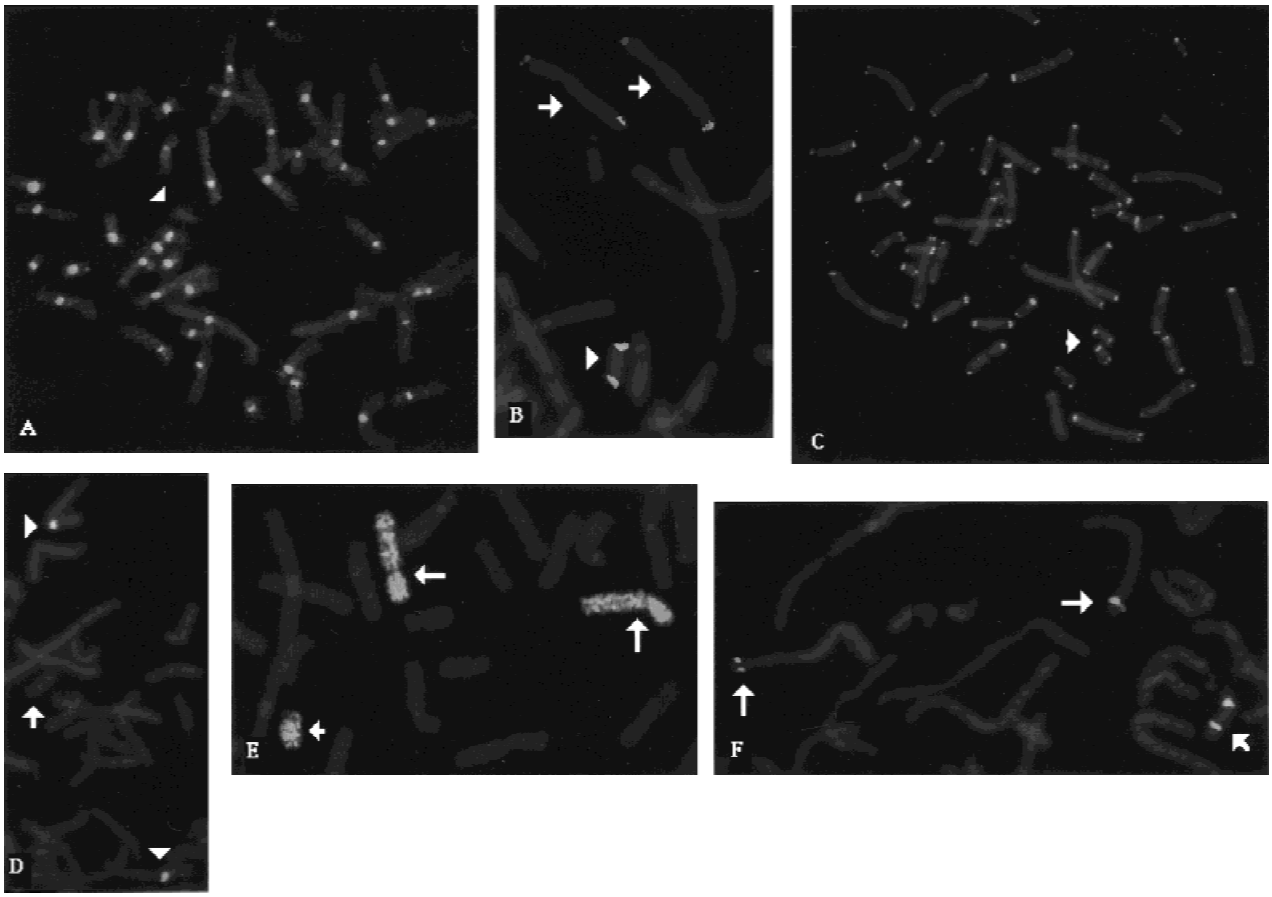

Fig. 3. FISH analyses. An arrow or arrowhead indicates the marker chromosome. **A:** Lack of signal on the marker with the all centromeric probe. **B:** Hybridization of the 8q subtelomeric probe on both ends of the marker. **C:** Hybridization of an all telomeric probe to both ends of the marker. **D:** Lack of signal on the marker with an 8-centromere probe (DS8Z1). **E:** The marker is painted uniformly with a coatasome 8 probe. **F:** Presence of two signals on the marker chromosome with a c-myc probe from 8q24.

1989]. However, hormone levels and imaging of their ovaries were not reported. Some of our patient's findings are potentially compatible with in utero alcohol exposure [Spohr et al., 1993].

The patient's myasthenia gravis is an antibody-mediated, autoimmune disorder. Prevalence is roughly 1 in 20,000 [Marx et al., 1997], and occurrence is usually sporadic. Familial occurrence is approximately 1%, but genetic factors have not been well defined [Evoli et al., 1995]. Genes for five subunits of the acetylcholine receptor have been mapped to chromosomes 2 and 17 [Lobos, 1993]. Thus, a relationship between tetrasomy 8q and myasthenia gravis is not known.

Stable analphoid marker chromosomes were first described by Callen et al. [1992] and Crolla et al. [1992], and at least 38 such markers with neocentromeres have been reported (Table I). Some chromosomes, like 13 and 15, appear to be more susceptible to forming analphoid markers, possibly due to differences in survival. Inversion duplication appears to be the most common type of analphoid marker, reported in 23 cases including our patient. Such duplications cause partial tetrasomy (cases 6–8, 10–13, 19–23, 26, 27–32, and 34 in Table I) or partial trisomy if accompanied by a deleted chromosome (cases 12, 17, 24, and 25 in Table I). Inversion duplications probably arise through a terminal deletion followed by U-type exchange. Other mechanisms for formation of analphoid markers include: internal excision of a centric fragment as a marker or ring chromosome, followed by recombination of the two fragments from either end [Maraschio et al., 1996; Voullaire et al., 1993; Wandall et al., 1998] and deletions [Depinet et al., 1997; Petit and Fryns, 1997].

A neocentromere has been identified on analphoid markers in the majority of cases. An exception is a Y-chromosome with an active neocentromere within the Yqh region and an inactive native centromere [Bukvic et al., 1996; Rivera et al., 1996; Tyler-Smith et al., 1999].

Most of these markers had telomeres at both ends (9/10), except on a deletion reported by Depinet et al. [1997] and a ring reported by Slater et al. [1999]. Even though the telomeres were absent, both markers were stable and present in 62% amniocytes and 80% fibroblasts, 97% lymphocytes and 100% fibroblasts, respectively.

Mosaicism involving the marker chromosome appeared to develop with age in our patient. At birth, all lymphocytes analyzed contained the marker, and the proportion declined to 75% at 16 years. Mosaicism in lymphocytes in published cases ranged from 5–98% (Table I). Fibroblasts and transformed lymphocytes

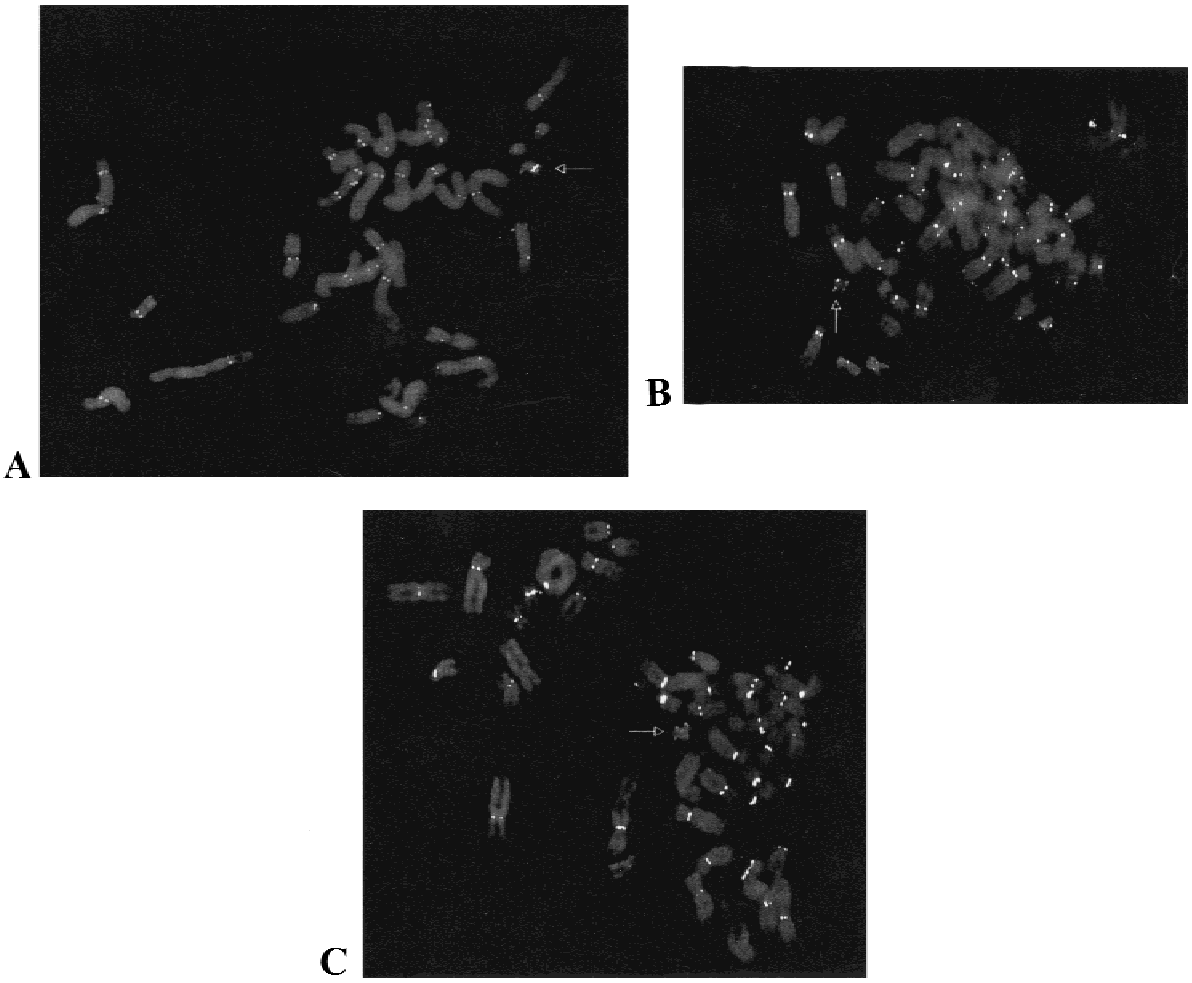

Fig 4. The marker chromosome (tagged with c-myc) was positive for centromeric proteins **(A)** CENP-C and **(B)** CENP-E, but negative for **(C)** CENP-B.

sometimes have a lower percentage—or absence—of the marker [e.g., three cases of lymphoblastoid transformed cultures reported by Blennow et al., 1994; Depinet et al., 1997]. Such findings may reflect mitotic errors or instability [Depinet et al., 1997]. Three chromosomes with neocentromeres were paternally inherited and hence stable through meiosis [Rivera et al., 1996; Tyler-Smith et al., 1999; Wandall et al., 1998].

The mechanism involved in the origin of these analphoid markers may be diverse. A paternal meiotic error was found in one case of dup(15)(qter→q25.3::q25.3→qter) [Depinet et al., 1997], and a postzygotic origin on the paternal chromosome in two additional cases [Depinet et al., 1997].

Centromere protein CENP-C (part of the kinetochore plates and required for kinetochore assembly; essential for mitosis) and CENP-E (a motor molecule that is important for chromosome movement) were present at the neocentromere. CENP-B (a functionally unknown centromere protein that binds to a subset of α-satellite DNA at active and inactive centromeres) was absent from the marker in this study. Similar results were observed in other markers studied using CENP antibodies (Table I). Recently, CENP-F (which associates transiently with the kinetochore, playing an apparent role in kinetochore maturation and signaling pathways for cell division) and INCENP (passenger protein) have broad localization along chromosomes in early mitosis but gradually become concentrated at the centromere as the cell progresses to mid metaphase. During metaphase/anaphase transition INCENPs remain confined to the metaphase plate, associating with stem body material, which coats the overlapping antiparallel microtubules of the central spindle, while sister chromatids migrate to the poles.) antibodies have been used to further define neocentromere activity [Voullaire et al., 1999; Slater et al., 1999].

The overall low occurrence of analphoid marker chromosomes suggests that rare events are involved in formation of neocentromeres. Epigenetic modification of chromatin is believed to be required, rather than specific DNA sequences [Fisher et al., 1997]. For example, the sequence of a neocentromere on mar del(10) showed only a loose resemblance to the sequences of known centromeres (Table I) [Barry et al., 1999; du Sart et al., 1997; Voullaire et al., 1993]. Sequence analysis of additional neocentromeres will be of considerable interest. The high frequency of inversion duplication among analphoid marker chromosomes suggests that palindromic sequences formed probably by the U-type ex-

TABLE I. Summary of Reported Markers With a Neocentromere*

Sl #	Karotype	Tissue distribution of marker (%)		Transformed lymphoblasts	Centromere protein		Reference
		Lymphocytes	Fibroblasts		CENP-B	Other	
1	47,XX,+mar	—	100	—	NA	NA	Crolla et al., 1992
2	47,XX,+mar	Mos	NA	NA	NA	NA	Callen et al., 1992
3	46,XY,r(1)(p32p36)	97	100	—	—	+ E, F & INCENP	Slanter et al., 1999
4	47,XY,del(2)(p11→p21), +der(2)(p11→[neocen]→p12)	NA	100	NA	NA	NA	Petit and Fryns, 1997
5	47,XX,−3,r(3)(p21.3→q25),+rea(3)(pter→p23[neocen]p23→p21.3::q25→qter)	100	100	NA	NA	NA	Marachio et al., 1996
6	47,XX,+inv dup(3)(qter→27.2::q27.2→[neocen]→qter)	Mos	NA	NA	–	+ C & E	Teshima et al., 1998
7	47,XY,+inv dup(3)(qter→27.2::q27.2→[neocen]→qter)	Mos	NA	NA	–	+ E	Teshima et al., 1998
8	46,XY,+inv dup(3)(qter→27.2::q27.1→[neocen]qter)	30	6	NA	NA	NA	Portnoi et al., 1999
9	46,XX,del(3)(pter→11::q11→q26[neocen]q26→qter) Father 47,XY,del(3)(p11q11),+mar	100	100 father	NA	NA	+ CREST	Wandall et al., 1998
10	de novo 47,XX,+der(8)(pter→p23.1::p23.1→[neocen]→pter)	100	NA	NA	NA	+ CREST	Ohashi et al., 1994
11	47,XX,+inv dup (8)(qter→q23.3::q23.3→q23.3→[neocen]→qter)/46.XX	55	NA	90	—	+ C & E	Present
12	47,XY,del(9)(p12),+der(9)(pter→p12::p12→[neocen]→pter)	100	NA	100	—	+ C & E	Vance et al., 1997
13	47,XY,+der(9)(pter→p21.2::p21.2→[neocen]→pter)	100	NA	NA	NA	NA	Depinet et al., 1997
14	48,XY,−10,+rdel(10)(p12..2→q23.3),+mar del(10)(pter→p12..2::q23.3→q25.2[neocen] q25.2→qter),+ bisatellited marker chromosome 10 abnormalities were de novo, and the bisatellited marker was paternally inherited.	100	100	100	—	+ CREST	Voullaire et al., 1993
15	dup(10)(qter→q11.2::p11.2→q26[neocen]q26→qter)	90 (bone marrow)	NA	NA	NA	NA	Abeliovich et al., 1996
16	47,XX,del(10)(q11→q23),+der(10)(q11→[neocen]→q23)/46,XX	62	80	NA	—	+ C & E	Depinet et al., 1997
17	47,XY,del(11)(q22),+der(11)(qter→q22::q22→[neocen]→qter)	100	100	100	—	+ C & E	Depinet et al., 1997
18	47,XY,+der(13)(q32→qter::q32→qter)	100	60	—	—	—	Zinn et al., 1993
19	47,XY,+der(13)(qter→q32::q32→[neocen]→qter)/46,XY	98	8	32	—	+ C & E	Depinet et al., 1997
20	de novo mos47,XX,+idic(13) (qter→q32[neocen]q32→qter)/48,XX,+idic(13)(qter→q32[neocen] q32→qter)×2/46 XX	75 [1 mar] 15 [2 mar]	—	—	—	—	Lozzio et al., 1997
21	47,+inv dup(13)(qter→q21.2::q21.1→q32[neocen]q32→qter)/46	Mos	NA	NA	—	+ C	Warburton et al., 1997a
22	47,XX,+inv dup(13)(qter→q21.3::q21.3→qter)/46,XX	49	—	—	—	—	Tohma et al., 1998
23	47,XY,+inv dup(13)(qter→q31::q31→qter)/46,XY	60	—	—	—	—	Tohma et al., 1998
24	47,XY,r(13)(q14),+dup(13)(qter→q14::q14→q21[neocen]q21→qter)	100	—	—	—	—	Dowhanick et al., 1998
25	de novo 47,XX,del(13)(pter→q21:),+iace (13)(qter→21.1::q21.1→32[neocen]q32→qter)	100	NA	NA	—	—	Rivera et al., 1999
26	47,XX,del(14)(q32.1→qter), +der(14)(qter→q32::q32→[neocen]→qter)	100	NA	100	NA	+ CREST	Sacchi et al., 1996
27	de novo 47,XY,+der(15)(qter→q23::q23[neocen]q23→qter)/46,XY	70	11	0	NA	NA	Blennow et al., 1994
28	de novo 47,XX,+der(15)(qter→q24::q24[neocen]q24→qter)/46,XX	80	NA	0	NA	NA	Blennow et al., 1994
29	de novo 47,XY,+i(15)(qter→q25::q25→qter)/46,XY	79	—	—	—	—	Van den Enden et al., 1996
30	47,XX,+der(15)(qter→q25.3::q25.3→[neocen]→qter)/46,XX	82	NA	33	—	+ C	Depinet et al., 1997
31	47,XY,+der(15)(qter→q25.3::q25.3→[neocen]→qter)/46,XY	74	NA	3	NA	NA	Depinet et al., 1997
32	47,XY,+der(15)(qter→q26.1::q26.1→[neocen]→qter)/46,XY	86	NA	0	NA	NA	Depinet et al., 1997
33	de novo 47,XX,+der(15)(qter→q25::q25→[neocen]→qter)	100	NA	NA	NA	NA	Huang et al., 1998
34	46,XY/46,XY,mar19/46,XY,dic mar19/46,XY,acemar 19	20	—	NA	NA	NA	Crolla et al., 1992
35	de novo 47,XX,del(20)(p11.2),+inv dup(20)(pter→p11.2::p11.2→p12[neocen]p12→pter)	100	100	100	—	+ A, C, E, F and INCENP	Voullaire et al., 1999
36	46,X,inv(Y)(pter→psucen→q12[neocen]q12→qter)pat	99	—	—	—	—	Rivera et al., 1996
37	45,X/46,XY/47,XY,+mar(Y)(pter→psucen→q12[neocen]q12→qter)	5	NA	NA	NA	NA	Bukvic et al., 1996
38	46,X,mar(Y) (pter→psucen→q12[neocen]q12→qter)pat	96 Father	100 Amniocytes	+	—	+ A, C, & E	Tyler-Smith et al., 1999

*NA, information not available.

change may direct epigenetic factors. A constellation of epigenetic factors have been shown to imprint a neocentromere in *Schizosaccharomyces pombe* [Karpen and Allshire, 1997].

Methods for identifying marker chromosomes are now fairly well established. Nonsatellited and C-band negative marker chromosomes should be tested for absence of alphoid sequences by use of an all centromere α-satellite probe to assess for neocentromeres. Chromosome painting probes can provide confirmation of the origin of the marker, if the karyotype gives a preliminary indication [Abeliovich et al., 1996; Vance et al., 1997]. Spectral karyotyping [Huang et al., 1998] and subtelomeric probes (present study) can be used for other, unidentifiable acentric markers. The simpler method is to use subtelomeric probes, when the acentric marker involves the telomeric region. Better resolution is obtained with subtelomeric probes followed by painting or spectral karyotyping, with >12 Mb resolving power [Schrock et al., 1996]. Reverse painting with the microdissected/flow-sorted marker as the probe [Blennow et al., 1994; Ohashi et al., 1994; Sacchi et al., 1996] and comparative genomic hybridization are mainly research methods at the current time and have lower resolution (4–10 Mb) [Levy et al., 1998]. When the mosaicism for an analphoid marker is assessed in fibroblasts useful data can be generated using FISH on interphases obtained after protein digestion of the skin biopsy prior to culture set up, to eliminate in vitro culture errors.

REFERENCES

Abeliovich D, Yehuda O, Ben-Neriah S, Kapelushnik Y, Ben-Yehuda D. 1996. Dup (10q) lacking alpha-satellite DNA in bone marrow cells of a patient with acute myeloid leukemia. Cancer Genet Cytogenet 89:1–6.

Barry AE, Howman EV, Cancilla MR, Saffery R, Choo KH. 1999. Sequence analysis of an 80 kb human neocentromere. Hum Mol Genet 8:217–227.

Bijlsma JB, Wijffels JC, Tegelaers WH. 1972. C8 trisomy mosaicism syndrome. Helv Paediatr Acta 27:281–298.

Blennow E, Telenius H, de Vos D, Larsson C, Henriksson P, Johansson O, Carter NP, Nordenskjold M. 1994. Tetrasomy 15q: two marker chromosomes with no detectable alpha-satellite DNA. Am J Hum Genet 54:877–883.

Buckton KE, Spowart G, Newton MS, Evans HJ. 1985. Forty four probands with an additional "marker" chromosome. Hum Genet 69:353–370.

Bukvic N, Susca F, Gentile M, Tangari E, Ianniruberto A, Guanti G. 1996. An unusual dicentric Y chromosome with a functional centromere with no detectable alpha-satellite. Hum Genet 97:453–456.

Callen DF, Eyre H, Yip MY, Freemantle J, Haan EA. 1992. Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. Am J Med Genet 43:709–715.

Caspersson T, Lindsten J, Zech L, Buckton KE, Price WH. 1972. Four patients with trisomy 8 identified by the fluorescence and Giemsa banding techniques. J Med Genet 9:1–7.

Cassidy SB, McGee BJ, Van Eys J, Nance WE, Engel E. 1975. Trisomy 8 syndrome. Pediatrics 56:826–831.

Choo KHA. 1997. Centromere DNA dynamics: latent centromeres and neocentromere formation. Am J Hum Genet 61:1225–1233.

Crolla JA, Dennis NR, Jacobs PA. 1992. A non-isotopic in situ hybridisation study of the chromosomal origin of 15 supernumerary marker chromosomes in man. J Med Genet 29:699–703.

Depinet TW, Zackowski JL, Earnshaw WC, Kaffe S, Sekhon GS, Stallard R, Sullivan BA, Vance GH, Van Dyke DL, Willard HF, et al. 1997. Characterization of neo-centromeres in marker chromosomes lacking detectable alpha-satellite DNA. Hum Mol Genet 6:1195–1204.

Dowhanick JJ, Celle L, Owens N, Lieber C, Aviv HA, Zackai EH, Spinner NB. 1998. Activation of a latent centromere following chromosome 13 rearrangement in a patient with bilateral retinoblastoma. Am J Hum Genet 63(suppl):A132.

Du Sart D, Cancilla MR, Earle E, Mao J, Saffery R, Tainton KM, Kalitsis P, Martyn J, Barry AE, Choo KHA. 1997. A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nat Genet 16:144–153.

Earnshaw WC, Ratie H III, Stetten G. 1989. Visualization of centromere proteins CENP-B and CENP-C on a stable dicentric chromosome in cytological spreads. Chromosoma 98:1–12.

Evoli A, Batocchi AP, Zelano G, Uncini A, Palmisani MT, Tonali P. 1995. Familial autoimmune myasthenia gravis: report of four families. J Neurol Neurosurg Psychiatry 58:729–731.

Faulkner NE, Vig B, Echeverri CJ, Wordeman L, Vallee RB. 1998. Localization of motor-related proteins and associated complexes to active, but not inactive, centromeres. Hum Mol Genet 7:671–677.

Fisher AM, Al-Gazali L, Pramathan T, Quaife R, Cockwell AE, Barber JC, Earnshaw WC, Axelman J, Migeon BR, Tyler-Smith C. 1997. Centromere inactivation in a dicentric human Y;21 translocation chromosome. Chromosoma 106:188–206.

Fryns JP, Verresen H, Van den Berghe H. 1974. Partial trisomy 8: trisomy of the distal part of the long arm of chromosome number 8+ (8q2) in a severely retarded and malformed girl. Humangenetik 24:241–246.

Govind A, Obhrai MS, Clayton RN. 1999. Polycystic ovaries are inherited as an autosomal dominant trait: analysis of 29 polycystic ovary syndrome and 10 control families. J Clin Endocrinol Metab 84:38–43.

Huang B, Ning Y, Lamb AN, Sandlin CJ, Jamehdor M, Ried T, Bartley J. 1998. Identification of an unusual marker chromosome by spectral karyotyping. Am J Med Genet 80:368–372.

Karpen GH, Allshire RC. 1997. The case for epigenetic effects on centromere identity and function. Trends Genet 13:489–496.

Levy B, Dun TM, Kaffe S, Kardon N, Hirschhorn K. 1998. Clinical application of comparative genomic hybridization. Genet Med 1:4–12.

Lobos EA. 1993. Five subunit genes of the human muscle nicotinic acetylcholine receptor are mapped to two linkage groups on chromosomes 2 and 17. Genomics 17:642–650.

Lozzio C, Lyall L, Bamberger E, Ralston E. 1997. Mosaic for small metacentric chromosome with tetrasomy 13q32-qter and non-alpha satellite centromere. Am J Hum Genet 61(suppl):A132.

Maraschio P, Tupler R, Rossi E, Barbierato L, Uccellatore F, Rocchi M, Zuffardi O, Fraccaro M. 1996. A novel mechanism for the origin of supernumerary marker chromosomes. Hum Genet 97:382–386.

Marx A, Wilisch A, Schultz A, Gattenlohner S, Nenninger R, Muller-Hermelink HK. 1997. Pathogenesis of myasthenia gravis. Virchows Arch 430:355–364.

Ohashi H, Wakui K, Ogawa K, Okano T, Niikawa N, Fukushima Y. 1994. A stable acentric marker chromosome: possible existence of an intercalary ancient centromere at distal 8p. Am J Hum Genet 55:1202–1208.

Page SL, Earnshaw WC, Choo KHA, Shaffer LG. 1995. Further evidence that CENP-C is a necessary component of active centromeres: studies of a dic(X;15) with simultaneous immunofluorescence and FISH. Hum Mol Genetics 4:289–294.

Petit P, Fryns JP. 1997. Interstitial deletion 2p accompanied by marker chromosome formation of the deleted segment resulting in a stable acentric marker chromosome. Genet Couns 8:341–343.

Portnoi MF, Boutchnei S, Bouscarat F, Morlier G, Nizard S, Dersarkissian H, Crickx B, Nouchy M, Taillemite J-L, Belaich S. 1999. Skin pigmentary anomalies and mosaicism for an acentric marker chromosome originating from 3q. J Med Genet 36:246–250.

Rivera H, Vassquez AI, Ayala-Madrigal ML, Ramirez-Duenas ML, Davalos IP. 1996. Alphoidless centromere of a familial unstable inverted Y chromosome. Ann Genet 39:236–239.

Rivera H, Vasquez AI, Garcia-Cruz D, Crolla JA. 1999. Neocentromere at 13q32 in one of two stable markers derived from 13q21 break. Am J Med Genet 85:385–388.

Romain DR, Bloxham RA, Columbano-Green LM, Chapman CJ, Parfitt RG, Smythe RH, Cairney H. 1989. Familial distal trisomy 8(q24.13→qter). J Med Genet 26:133–138.

Sacchi N, Magnani I, Fuhrman-Conti AM, Monard SP, Darfler M. 1996. A stable marker chromosome with a cryptic centromere: evidence for centromeric sequences associated with an inverted duplication. Cytogenet Cell Genet 73:123–129.

Sanchez O, Yunis JJ. 1974. Partial trisomy 8(8q24) and the trisomy-8 syndrome. Humangenetik 23:297–303.

Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, et al. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273: 494–497.

Slater HR, Nouri S, Earle E, Lo AW, Hale LG, Choo KH 1999. Neocentromere formation in a stable ring 1p32-p36.1 chromosome. J Med Genet 36:914–918.

Spohr HL, Willms J, Steinhausen HC. 1993. Prenatal alcohol exposure and long-term developmental consequences. Lancet 341:907–910.

Stengel-Rutkowski S, Lohse K, Herzog C, Apacik C, Couturier J, Albert A, Belohradsky B. 1992. Partial trisomy 8q. Two case reports with maternal translocation and inverted insertion: phenotype analyses and reflections on the risk. Clin Genet 42:178–185.

Sullivan BA, Schwartz S. 1995. Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. Hum Mol Genetics 4: 2189–2197.

Taylor AE. 1998. Polycystic ovary syndrome. Endocrinol Metab Clin North Am 27:877–902.

Teshima I, Bawle E, Weksberg R, Shuman C, Van Dyke DL, Schwartz S. 1998. Acentric 3qter markers. Am J Hum Genet 63(suppl):A152.

Tohma T, Ohashi H, Hasegawa T, Nagai T, Fukushima Y, Naritomi K. 1998. Two cases of mosaic partial tetrasomy 13q associated with an acentric marker chromosome. Am J Hum Genet 63(suppl):A152.

Tyler-Smith C, Gimelli G, Giglio S, Floridia G, Pandya A, Terzoli G, Warburton PE, Earnshaw WC, Zuffardi O. 1999. Transmission of a fully functional human neocentromere through three generations. Am J Hum Genet 64:1440–1444.

Vafa O, Sullivan KF. 1997. Chromatin containing CENP-A and α-satellite DNA is a major component of the inner kinetochore plate. Curr Biol 7:897–900.

Vance GH, Curtis CA, Heerema NA, Schwartz S, Palmer CG. 1997. An apparently acentric marker chromosome originating from 9p with a functional centromere without detectable alpha and beta satellite sequences. Am J Med Genet 71:436–442.

Van den Enden A, Verschraegen-Spae MR, Van Roy N, Decaluwe W, De Praeter C, Speleman F, 1996. Mosaic tetrasomy15q25→qter in a newborn infant with multiple anomalies. Am J Med Genet 63:482–485.

Voullaire LE, Slater HR, Petrovic V, Choo KHA. 1993. A functional marker centromere with no detectable alpha-satellite, satellite III, or CENP-B protein: activation of a latent centromere? Am J Hum Genet 52:1153–1163.

Voullaire LE, Saffery R, Davies J, Earle E, Kalitsis P, Slater H, Irvine DV, Choo KHA. 1999. Trisomy 20p resulting from inverted duplication and neocentromere formation. Am J Med Genet 85:403–408.

Wandall A, Tranebjaerg L, Tommerup N. 1998. A neocentromere on human chromosome 3 without detectable α-satellite DNA forms morphologically normal kinetochores. Chromosoma 107:359–365.

Warburton D, Perricone L, Say B, Carpenter N, Yu C-Y, Warburton PE. 1997a. Inv dup(13)(qterq21.1) with neocentromere in 13q32: precise definition of duplication and centromere location using CENP antibodies and FISH. Am J Hum Genet 61(suppl):A142.

Warburton PE, Cooke CA, Bourassa S, Vafa O, Sullivan BA, Stetten G, Gimelli G, Warburton D, Tyler-Smith C, Sullivan KF, et al. 1997b. Immunolocalization of CENP-A suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. Curr Biol 7:901–904.

Zinn AB, Rush PW, Liebman J, Stallard R, Schwartz S. 1993. Identification of an unusual marker chromosome lacking alpha satellite DNA. Am J Hum Genet 53(suppl):A633.